Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

Value and Challenges: Nucleic Acid Amplification Tests for SARS–CoV-2 in Hospitalized COVID-19 Patients

Dear editor,

We read with the interest the recent paper by Ma *et al.* who described the viral dynamics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) across a spectrum of disease severity in Coronavirus disease 2019 (COVID-19) in a prospective cohort study.[1] To date, most countries have confirmed COVID-19 cases of person-to-person spread, and the number of confirmed cases worldwide is expected to continue to increase. It has been emphasized that diagnostic testing for SARS-CoV-2 was an especially important tool in the diagnosis and management of patients with COVID-19.[2] Moreover, the specificity and negative predictive value of SARS-CoV-2 testing have not been systematically evaluated. Therefore, the clinical sampling frequency for inpatients with COVID-19 should also be carefully evaluated. It is of great importance to evaluate the values and challenges of nucleic acid amplification tests for SARS–CoV-2 in hospitalized COVID-19 patients. At present, China has brought outbreaks under control with draconian measures and most patients have discharged from the hospital. Here, we reported the current status of RNA-based diagnostic testing and the pattern of viral infection and clearance in hospitalized patients, providing evidence for test utilization and diagnostic stewardship of SARS-CoV-2 tests.

We included 3232 consecutive patients with COVID-19 who were hospitalized between January 18th and March 27th, 2020 (data cutoff date) at Tongji Hospital, a designated hospital for severe COVID-19 patients in Wuhan, China. All patients included in the present study were verified as positive for SARS-CoV-2 infection by reverse transcriptase polymerase chain reaction (RT-PCR). The specific operation methods were followed according to the instructions and were consistent with other literature.[3,4] According to the COVID-19 diagnosis and treatment plan issued by the National Health Commission, all patients included were diagnosed as moderate to severe cases. Clinical data were collected from medical records. The Ethical Committee of Tongji Hospital of Tongji Medical College at Huazhong University of Science and Technology approved this study (TJ-IRB20200311). Written informed consent was not obtained because the data were analyzed retrospectively and anonymously.

As of March 27th 2020, 3075 of these patients had at least one RT-PCR test during hospitalization, contributing 12 110 results. In total, 10 309 oropharyngeal swabs (OP) from 3003 patients and 1141 nasopharyngeal swabs (NP) from 567 patients were tested. In addition, there were 660 specimens by other sampling methods (e.g., bronchoalveolar lavage fluid, anal swabs) being collected and tested. The overall positive rate of NP was 18.1% (207/1141), which was higher than that of OP (16.7%, 1718/10 314). The positive rates also differed between patients who were died and discharged (37.0% vs. 16.0%). It should be noted that only 42.5% of death cases (62/146) were tested positive in the last RT-PCR test before death. The average intervals between two viral tests during hospital stay were 6.2 days for death cases, with 6.0 days for survivors. Currently, the US CDC recommended collecting only NP,[5] while current public health England guidance advises samples from the upper respiratory tract should be sought as NP, OP, or both in combination.[6] In the present study, the overall positive rate of NP was higher than that of OP. We also evaluated the proportion of false-negative results (negatives between two positive results during hospitalization) among all negative results.[7] The false-negative rate of OP was 10.0% (863/8596), while NP was 8.4% (78/934). However, three patients have contributed 33 false-negative oropharyngeal swabs (33/78), indicating significant individual bias. Here we suggested that the nasopharyngeal specimen is the preferred choice for swab-based SARS-CoV-2 testing with higher sensibility and specificity. Moreover, the negative predictive value of viral tests should be carefully evaluated. At present clinical practice, patients with improved respiratory symptoms, improved pulmonary imaging, and nucleic acid tests negative twice consecutively (sampling interval $\geq$ 24 hours) can be discharged. However, the data showed that people can test positive for the virus even after two consecutive negative results. Pan et al. reported that potential false-negative nucleic acid testing results for SARS-CoV-2 could be caused by thermal inactivation of samples with low viral loads.[8] According to our study, repeated viral RT-PCR testing separated by prolonged duration is needed for viral clearance evaluation. Other immunological parameters or antibody test should also be used in combined with RT-PCR negative test. Negative results must be interpreted with clinical observations, patient history, and epidemiological information.

For 2876 survivors, SARS-CoV-2 infection persistence curves were generated based on Kaplan-Meier analysis (Fig. 1). The median duration from onset of symptoms to pathogens clearance was 24 days (IQR 17-33). The median duration from hospital admission to pathogens clearance was 8 days (IQR 3-14). For patients with reliable pre-admission pathogens-identified records, the median duration from pathogens identified to pathogens clearance was 16 days (IQR 11-24). Generally, it takes a person several days to weeks to show symptoms after being exposed to the virus. Our analysis indicated that the median duration from onset of symptoms to hospital admission was 16 days in Wuhan, China. The clinical sampling frequency for inpatients with COVID-19 should be based on coronavirus infection and clearance pattern. We also suggested that the persistent positive SARS-CoV-2 was associated with worse prognosis, while negative viral tests could not indicate improvements of diseases. Since positive results are indicative of active infection, further studies are required to confirm the prognostic and predictive value of positive results in inpatients.

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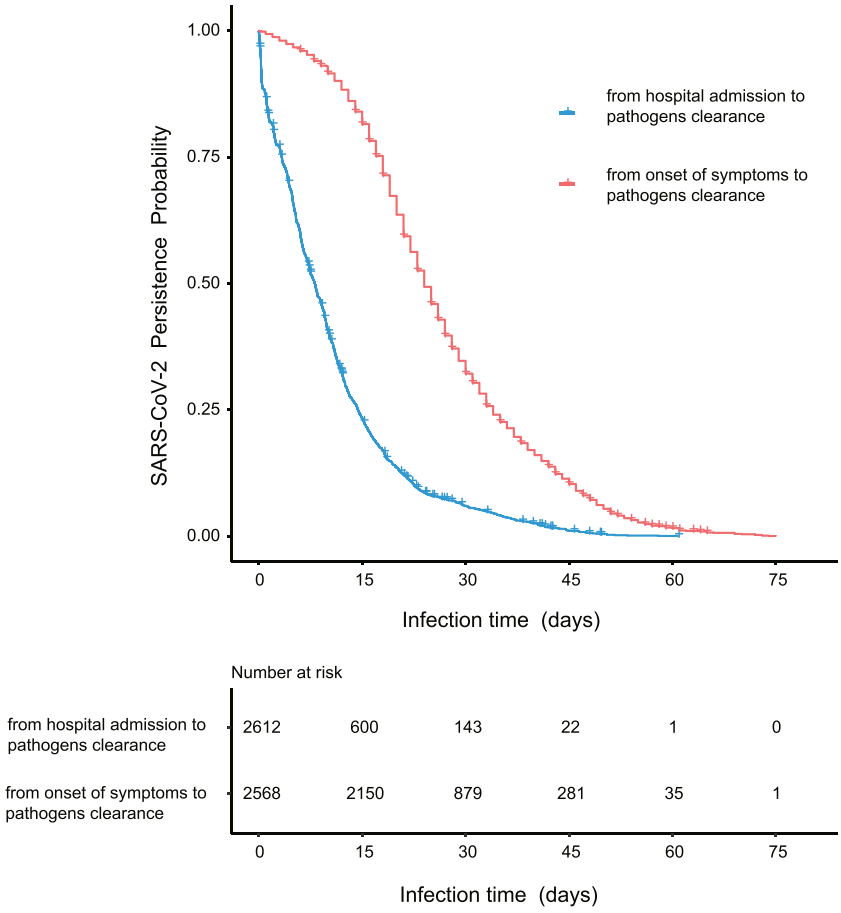

Fig. 1. SARS-CoV-2 infection persistence curves for survivors.

In summary, appropriate and efficient standards for testing are of great significance to prevent the sustained spread of COVID-19 and monitor disease progression for patients. Therefore, this study highlights the following observations: the nasopharyngeal specimen is the preferred choice for swab-based SARS-CoV-2 testing with higher specificity; the negative predictive value of viral tests should be carefully evaluated; the persistent positive SARS-CoV-2 was associated with worse prognosis, while negative viral tests could not indicate improvements of diseases. Most importantly, the analyses in the current study indicated that the clinical sampling frequency for hospitalized patients with COVID-19 should be based on coronavirus infection and clearance pattern.

Author Contributions

Gang Chen, Peng Wu and Chaoyang Sun conceived and designed the study; Yifan Meng, and Ensong Guo performed the statistical analysis; Yifan Meng, Ensong Guo, Jia Liu and Xiaoyuan Huang drafted the article; Yifan Meng, Ensong Guo, Jia Liu and Xiaoyuan Huang, Gang Chen, Peng Wu and Chaoyang Sun contributed to the data collection and quality control; Gang Chen, Peng Wu and Chaoyang Sun made critical revisions to the manuscript.

Declaration of Competing Interest

All authors declare that they have no financial or other conflicts of interest.

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Yifan Meng, Ensong Guo, Jia Liu, Xiaoyuan Huang, Chaoyang Sun**, Peng Wu**, Gang Chen*
National Clinical Research Center of Gynecology and Obstetrics, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China
Cancer Biology Research Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

*Corresponding author at: National Clinical Research Center of Gynecology and Obstetrics, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
E-mail addresses: suncydoctor@gmail.com (C. Sun), pengwu8626@tjh.tjmu.edu.cn (P. Wu), tjchengang@hust.edu.cn (G. Chen)